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Some effects of chlorfenvinphos, an organophosphorus insecticide, on populations of soil animals

PAR

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Chlorfenvinphos [Birlane **, diethyl 1-(2',4'-dichlorophenyl)-2-chlorovinyl phosphate, previously known as compound SD 7859 and GC 4072] has shown great promise for controlling wheat bulb fly [*Leptohylemyia coarctata* (Fall.)], cabbage root fly [*Erioischia brassicae* (Bouche)] and other dipterous pests. Its residues in soil have an initial half-life of from 2-23 weeks depending on the type of soil, formulation and dosage [BEYNON *et al.*, 1966 (b)].

When pesticides are applied to soils, they may affect species of the soil fauna other than the pests that are to be controlled. The present work describes the evaluation of the possible side-effects of chlorfenvinphos on soil fauna. Whilst chlorfenvinphos is likely to be a little more persistent in soils than many other organophosphorus insecticides, it is far less persistent than chlorinated hydrocarbons such as dieldrin and DDT. It has been shown, however, (EDWARDS, 1965) that even transient chemicals such as fumigants can disturb the soil population balance for several years, if their effects are drastic.

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EXPERIMENTAL METHODS

a) **Field experiments.**

i) *Plot sizes and application rates.*

Four experiments were used to assess changes in populations of micro-arthropods in soil treated with clorfenvinphos. In two experiments, potatoes were planted on newly ploughed pasture. In the other two experiments, spring wheat was sown, also on newly ploughed pasture. Untreated strips 3 feet wide were left between plots. The insecticides were either sprayed on the soil surface as a diluted emulsifiable concentrate (EC) or applied as granules. The insecticide was rotovated into the soil as evenly as possible. There were four replications of each treatment and control. Table I gives details of the insecticides and sites. Throughout the text, all application rates are given in terms of lb of active ingredient per acre.

TABLE 1

Trial Sites and Application Rates

Site	Soil type	Size of plot (ft)	Crop	Insecticide		Date of application
				Formulation	Dose lb/ac	
Thursley	Sandy loam	20 × 10	Potatoes	50 % EC	8	13th April, 1965
Alton	Loam over clay	20 × 10	Potatoes	50 % EC	4	13th April, 1965
Woodstock	Clay loam with flints	12 × 12	Spring wheat	50 % EC 10 % granules	8 8	15th November, 1965
Bethersden	Clay loam	12 × 12	Spring wheat	50 % EC 50 % EC 10 % granules	4 8 8	17th March, 1966

ii) *Description of sites.*

1. *Thursley* (Kettleborough Farm, Surrey). The plots lay on an exposed, well-drained ridge of land. There was a large population of wireworms, a moderate population of micro- and macro-arthropods, and a small population of earthworms.

2. *Alton* (Manor Farm, Hampshire). The experimental area was enclosed and level. The wireworms exceeded 1,000,000 per acre and there was a large population of earthworms. The population of micro- and macro-arthropods was large and varied.

3. *Woodstock Agricultural Research Centre* (Sittingbourne, Kent). The field had been pasture for more than ten years. It had very many wireworms and earthworms and a large and diverse population of arthropods. There were more centipedes and millipedes than at the other sites. When the wheat had been harvested in the middle of August 1966, the plots became grassed-over.

4. *Bethersden* (Vine Farm, Kent). The field had a high water table and had been pasture for many years. The numbers of most animals, including wireworms

and earthworms, were larger than in the other experiments. Considerable numbers of chafer grubs were present. The plots were on the side of the field and, although poorly drained, did not become waterlogged. As at Woodstock, the plots became grassed-over after the crop had been harvested at the beginning of September.

iii) *Sampling, extraction and analysis.*

Four soil samples, each two inches in diameter, were taken to a depth of six inches from each plot once a month and the animals were extracted in modified Tullgren funnels immediately on return to the laboratory. The sample filled the sieve to a depth of approximately two inches and the soil surface was kept at approximately 30° C. All the Tullgren funnels were in a room at a constant temperature of 20° C, giving a gradient through the soil of 10° C, and extraction was usually complete in three days. The animals were then stored in a mixture of 70 % (v) aqueous alcohol and glycerol (in the ratio 20 : 1) until sorted under a binocular microscope.

Eight other soil samples, each one-inch in diameter, were taken to a depth of four inches from each plot to estimate the amount of insecticide remaining. The samples from replicates of the same treatment were bulked, mixed thoroughly, and the residues extracted from sub-samples by mixing them with anhydrous sodium sulphate and tumbling them in a 20 % acetone in hexane solution [BEYNON *et al.*, 1966 a)]. Residues were determined by gas-liquid chromatography using a Shandon Universal gas chromatograph fitted with an electron capture detector of a design similar to that described by Lovelock (1961).

The two-inch diameter cores were too small to assess changes in numbers of macro-arthropods and earthworms and these were counted using pitfall traps placed in the centre of each plot.

Earthworms and macro-arthropods were also estimated from three, two-feet square, quadrats at the end of each plot. The soil in the top three to four inches was hand-sorted and all macro-arthropods and earthworms counted. The bottom of the shallow pit was then levelled and one gallon of dilute formalin (25 ml formalin in 1 gallon water) was poured on. Earthworms and other animals that came to the surface were counted. This method involved destructive sampling of quite a large area of each plot and so was done only once in each experiment between two and five months after applying the insecticide.

b) Experiments in boxes out of doors.

The data on earthworms in the field were substantiated by experiments in the laboratory. Peat and sandy loam from East Anglia and a medium loam from Woodstock were sieved to remove large stones and worms and then used as media for the worms. Chlorfenvinphos was added as a 50 % EC diluted with water to correspond to 6 ppm of the wet weight of the soils. The soil was thoroughly mixed in a concrete mixer and pressed down into wooden boxes to a depth of 2 feet. The boxes were 9 × 9 inches in section, 2 1/2 feet deep, with metal gauze bottoms and tops closed with removable gauze lids. Nine such boxes prepared with chlorfenvinphos-treated soils and nine boxes with untreated soils were buried outdoors with the surface of the soil in the boxes level with the surface of the earth around them.

Within hours after filling the boxes, fourteen freshly obtained worms were added to each box, seven selected from deep-living species *(Allolobophora longa* and *Lumbricus terrestris)* and seven from shallower-living species *(A. caliginosa* and *A. rosea)*. They were covered by an inch of the corresponding soil. The total rainfall during the trial was 11.3 inches compared to 4.3 inches in 1964 during the same period, and the soil remained moist because the rain could enter the boxes.

TABLE 2

Acarina

Site	Date of sampling	Animal count as mean log of samples transformed to log (n + 1)					Standard error	Variance ratio (F)
		Untreated control plot	Plots treated as indicated					
			gran. 4 lb/ac	4 lb/ac EC	8 lb/ac gran.	8 lb/ac EC		
(1) *Trombidiformes*								
ALTON	17.5.65	0.435		0.169			0.0761	6.25*
	26.8.65	0,782	0.513	0.315			0.0505	8.98**
	13.10.65	0.772		0.821			0.0524	0.26
THURSLEY	17.5.65	0.825				0.993	0.0602	1.80
	23.8.65	1.496			1.794	1.449	0.0769	5.40**
	12.10.65	1.414				1.138	0.1168	3.62
WOODSTOCK	13.12.65	0.474			0.291	0.531	0.0421	2.03
	27.1.66	0.749			0.647	0.38	0.1276	5.88**
	3.3.66	0.465			0.23	0.651	0.1041	7.13**
	31.3.66	0.955			0.736	0.634	0.1137	4.07*
	28.4.66	1.096			0.676	0.68	0.0951	13.06**
	16.6.66	1.654			1.165	1.148	0.0869	27.60**
	11.8.66	1.224			1.121	1.095	0.0509	1.02
	3.10.66	1.503			1.03	1.279	0.0705	7.39**
	7.11.66	0.994			1.004	1.033	0.1034	0.04
(2) *Gamasina except Rhodacaridae*								
ALTON	17.5.65	0.93		0.232			0.0593	52.76**
	26.8.65	1.136	0.714	0.721			0.0628	11.00**
	13.10.65	1.036		1.025			0.0716	0.02
THURSLEY	17.5.65	1.015				0.084	0.0554	111.60**
	23.8.65	0.988			0.214	0.397	0.0395	35.10**
	12.10.65	1.048				0.748	0.0187	6.90*
WOODSTOCK	13.12.65	0.76			0.317	0.329	0.1026	9.50**
	27.1.66	0.799			0.448	0.533	0.0711	3.07
	3.3.66	0.788			0.473	0.478	0.1105	3.27*
	31.3.66	0.912			0.71	0.675	0.1631	1.82
	28.4.66	0.943			0.751	0.711	0.1123	2.10
	16.6.66	1.115			0.832	0.975	0.0618	4.88*
	11.8.66	1.057			1.083	0.912	0.0834	1.61
	3.10.66	1.041			1.000	1.033	0.0576	0.11
	7.11.66	0.95			0.876	0.859	0.0611	0.34
(3) *Rhodacaridae*								
ALTON	17.5.65	0.157		0.056			0.0469	1.39
	26.8.65	0.606	0.105	0.019			0.0781	20.21**
	13.10.65	0.336		0.218			0.075	1.14
THURSLEY	17.5.65	0.379				0.298	0.0835	0.63
	23.8.65	0.846			0.516	0.602	0.0441	3.05
	12.10.65	0.975				0.525	0.0758	13.11**
WOODSTOCK	13.12.65	0.509			0.348	0.584	0.1052	2.22
	27.1.66	0.607			0.248	0.411	0.0768	4.41*
	3.3.66	0.692			0.343	0.602	0.0922	5.43**
	31.3.66	0.72			0.427	0.682	0.0868	6.34**
	24.4.66	1.007			0.497	0.675	0.0852	20.93**
	16.6.66	1.225			0.894	0.781	0.0673	11.17**
	11.8.66	1.271			1.166	1.243	0.0607	0.76
	3.10.66	1.158			1.01	1.215	0.0447	2.99
	7.11.66	1.214			1.173	1.025	0.0718	2.58

Acarina *(continued)*

Site	Date of sampling	Animal count as mean log of samples transformed to log (n + 1)					Standard error	Variance ratio (F)
		Untreated control plot	Plots treated as indicated					
			4 lb/ac gran.	4 lb/ac EC	8 lb/ac gran.	8 lb/ac EC		
(4) *Tyroglyphidae*								
ALTON	17.5.65	0.075		0.067			0.056	0.02
	26.8.65	0.113	0.18	0.154			0.0831	0.53
THURSLEY	17.5.65	0.067				0.105	0.0344	0.38
	23.8.65	0.294			0.06	0.154	0.0512	1.67
	12.10.65	0.348				0.225	0.0494	0.87
WOODSTOCK	13.12.65	0.26			0.364	0.429	0.0586	0.82
	27.1.66	0.197			0.252	0.47	0.1052	2.93
	3.3.66	0.138			0.381	0.419	0.0768	2.53
	31.3.66	0.258			0.414	0.267	0.0845	1.01
	28.4.66	0.179			0.391	0.304	0.1181	1.87
	16.6.66	1.238			0.81	0.858	0.0802	10.84**
	11.8.66	0.686			0.728	0.694	0.0702	0.10
	3.10.66	0.623			0.402	0.652	0.0752	3.06
	7.11.66	0.892			0.741	0.689	0.0886	2.48
(5) *Oribatidae*								
ALTON	17.5.65	1.45		1.028			0.1405	21.23**
	26.8.65	1.738	1.479	1.429			0.1501	3.03
	13.10.65	1.329		1.748			0.0953	16.62**
THURSLEY	17.5.65	1.112				1.404	0.0983	10.40**
	23.8.65	1.044			1.736	1.555	0.0987	20.22**
	12.10.65	1.046				1.281	0.0938	3.71*
WOODSTOCK	13.12.65	1.003			0.812	1.117	0.1206	1.85
	27.1.66	0.947			1.128	1.142	0.1192	1.18
	3.3.66	0.936			1.052	1.267	0.0979	2.90
	31.3.66	1.15			1.446	1.185	0.1435	3.33*
	28.4.66	1.328			1.194	1.179	0.0775	1.18
	16.6.66	1.179			1.129	1.156	0.1073	0.14
	11.8.66	1.121			1.078	1.037	0.1521	0.21
	3.10.66	0.968			0.839	0.85	0.1523	0.61
	7.11.66	1.238			0.913	0.733	0.0933	4.14*

* Significant at 5 % level.
** Significant at 1 % level.

All the soil and worms in one treated and one untreated box, for each type of soil, were examined at 4, 10 and 21 weeks after chlorfenvinphos was applied. The soils were extracted with 20 % (v) acetone-hexane in the presence of anhydrous sodium sulphate. The worms were washed with water and kept alive moistened with water for 2-4 days in beakers to expel the earth from their alimentary canals. They were then washed, deep-frozen, chopped-up and weighed while frozen, and their tissues were macerated with 2 ml of 30 % (v) acetone-hexane and 1 g of anhdrous sodium sulphate for every gram of tissue, as described for crops [BEYNON *et al.*, 1966 (a)].

The soil and worm extracts were shaken with an equal volume of water to remove the acetone and hexane, dried with sodium sulphate and analysed for chlorfenvinphos by gas-liquid chromatography. The results, summarised in Table 15, are corrected for the blank values of less than 0.02 ppm for the untreated control

TABLE 3

Collembola

Site	Date of sampling	Animal count as mean log of samples transformed to log (n + 1): Untreated control plot	Plots treated as indicated: 4 lb/ac gran.	4 lb/ac EC	8 lb/ac gran.	8 lb/ac EC	Standard error	Variance ratio (F)
(1) *Onychiuridae*								
ALTON	17.5.65	0.37		0.019			0.0505	13.90**
	26.8.65	1.172	0.585	0.557			0.1218	13.94**
	13.10.65	0.98		1.221			0.0989	5.52**
THURSLEY	17.5.65	0.288				0.135	0.0459	2.70
	23.8.65	0.623			0.538	0.628	0.048	0.27
	12.10.65	1.143				1.379	0.1781	1.90
WOODSTOCK	13.12.65	0.889			0.999	0.855	0.0719	0.56
	27.1.66	1.046			0.7	0.872	0.0968	2.88
	3.3.66	0.899			0.607	0.782	0.0761	2.78
	31.3.66	1.082			0.962	1.117	0.1144	0.87
	28.4.66	1.19			0.864	1.027	0.1546	4.62**
	16.6.66	1.042			0.742	0.804	0.0826	4.22**
	11.8.66	1.43			1.396	1.282	0.1827	1.38
	3.10.66	1.445			1.428	1.405	0.1705	0.06
	7.11.66	1.7			1.569	1.543	0.0919	1.01
(2) *Isotomidae*								
ALTON	17.5.65	0.124		0.124			0.0431	0.00
	26.8.65	0.63	0.262	0.363			0.0619	6.65**
	13.10.65	0.786		0.555			0.0186	5.45**
THURSLEY	17.5.65	0.234				0.063	0.0487	2.86
	23.8.65	0.805			0.210	0.453	0.0499	16.85**
	12.10.65	0.903				0.885	0.1194	0.01
WOODSTOCK	13.12.65	0.324			0.191	0.330	0.0765	1.05
	27.1.66	0.225			0.154	0.3	0.0471	0.97
	3.3.66	0.677			0.298	0.429	0.0859	3.07
	31.3.66	1.195			0.503	0.768	0.1606	16.71**
	28.4.66	1.526			0.852	0.697	0.0848	25.32**
	16.6.66	1.616			1.163	1.241	0.0689	9.42**
	11.8.66	1.372			1.229	0.937	0.1013	8.88**
	3.10.66	1.436			1.175	1.241	0.0925	3.58*
	7.11.66	1.471			1.253	1.309	0.0922	3.36*
(3) *Entomobryidae*								
ALTON	17.5.65	0.075		0.086			0.0374	0.04
	26.8.65	0.949	0.861	0.717			0.0701	1.83
	13.10.65	1.316		1.519			0.1159	4.32*
THURSLEY	17.5.65	0.019				0.038	0.0255	0.33
	23.8.65	0.108			0.067	0.019	0.0492	1.17
	12.10.65	0.452				0.398	0.1043	0.13
WOODSTOCK	13.12.65	0.049			0.097	0.124	0.0514	0.52
	27.1.66	0.155			0.067	0.056	0.0445	1.39
	3.3.66	0.15			0.097	0.038	0.0372	1.35
	31.3.66	0.056			0.205	0.097	0.0828	2.56
	28.4.66	0.124			0.183	0.075	0.0594	0.99
	16.6.66	0.573			0.454	0.415	0.1053	0.95
	11.8.66	0.559			0.894	0.901	0.1421	7.82**
	3.10.66	0.84			0.978	1.099	0.1069	4.66*
	7.11.66	0.766			0.835	0.935	0.0893	1.07

Collembola *(continued)*

Site	Date of sampling	Animal count as mean log of samples transformed to log (n + 1)					Standard error	Variance ratio (F)
		Untreated control plot	Plots treated as indicated: 4 lb/ac gran.	4 lb/ac EC	8 lb/ac gran.	8 lb/ac EC		
(4) *Sminthuridae*								
ALTON	26.8.65	0.157	0.184	0.191			0.0551	0.10
	13.10.65	0.119		0.097			0.034	0.08
THURSLEY	12.10.65	0.067				0.03	0.0199	0.63
WOODSTOCK	13.12.65	0.049			0.049	0.197	0.0422	2.30
	27.1.66	0.427			0.105	0.235	0.1035	4.38*
	3.3.66	0.592			0.397	0.447	0.061	0.88
	31.3.66	0.848			0.658	0.616	0.0794	2.70
	28.4.66	0.525			0.533	0.404	0.1152	1.42
	16.6.66	0.278			0.221	0.216	0.0853	0.30
	11.8.66	0.614			0.488	0.469	0.065	1.66
	3.10.66	1.002			0.734	0.807	0.0885	3.21
	7.11.66	0.651			0.427	0.505	0.0581	2.03

* Significant at 5 % level.
** Significant at 1 % level.

TABLE 4

Myriapoda

Site	Date of sampling	Animal count as mean log of samples transformed to log (n + 1)					Standard error	Variance ratio (F)
		Untreated control plot	Plots treated as indicated: 4 lb/ac gran.	4 lb/ac EC	8 lb/ac gran.	8 lb/ac EC		
(1) *Symphyla*								
ALTON	17.5.65	0.03		0.177			0.0371	4.22
	26.8.65	0.019	0.03	0.067			0.036	0.79
WOODSTOCK	13.12.65	0.086			0.075	0.086	0.0342	0.03
	27.1.66	0.03			0.056	0.143	0.0193	2.39
	31.3.66	0.067			0.086	0.217	0.0484	2.77
	28.4.66	0.169			0.075	0.21	0.0343	1.70
	16.6.66	0.543			0.437	0.743	0.0652	2.88
	11.8.66	0.54			0.693	0.543	0.0786	1.91
	3.10.66	0.42			0.362	0.559	0.0711	2.78
	7.11.66	0.485			0.419	0.343	0.0925	1.35
(2) *Pauropoda*								
WOODSTOCK	13.12.65	0.18			0.056	0.086	0.0507	1.53
	27.1.66	0.049			0.075	0.135	0.0416	1.22
	3.3.66	0.46			0.116	0.21	0.0689	9.34**
	31.3.66	0.27			0.419	0.401	0.0899	1.99
	28.4.66	0.521			0.158	0.162	0.0554	9.44**
	16.6.66	0.78			0.195	0.556	0.1086	12.65**
	11.8.66	0.34			0.218	0.295	0.0782	0.85
	3.10.66	0.227			0.169	0.245	0.0726	0.38
	7.11.66	0.259			0.229	0.317	0.0447	0.53

** Significant at 1 % level.

TABLE 5

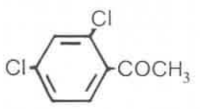
Annelida

Site	Date of sampling	Animal count as mean log of samples transformed to log (n + 1)					Standard error	Variance ratio (F)
		Untreated control plot	Plots treated as indicated					
			4 lb/ac gran.	4 lb/ac EC	8 lb/ac gran.	8 lb/ac EC		
(1) *Enchytraeidae*								
ALTON	17.5.65	0.318		0.210			0.0519	1.73
	26.8.65	0.235	0.281	0.284			0.0839	0.15
	13.10.65	0.124		0.135			0.0438	0.03
THURSLEY	17.5.65	0.385				0.564	0.0494	4.17
	23.8.65	0.18			0.304	0.322	0.1097	1.16
	12.10.65	0.094				0.355	0.0981	5.25*
WOODSTOCK	13.12.65	0.34			0.452	0.301	0.0622	1.24
	27.1.66	0.456			0.571	0.625	0.655	1.06
	3.3.66	0.597			0.799	0.745	0.097	1.62
	31.3.66	0.504			0.833	0.710	0.1219	5.08*
	28.4.66	0.758			1.112	0.959	0.0883	8.46**
	16.6.66	0.413			0.362	0.365	0.0747	0.27
	11.8.66	0.421			0.281	0.439	0.0937	1.52
	3.10.66	0.306			0.227	0.289	0.0551	0.48
	7.11.66	0.455			0.571	0.462	0.1039	1.08
(2) *Lumbricidae*								
ALTON	17.5.65	0.132		0.105			0.0301	0.17
	13.10.65	0.049		0.038			0.0419	0.06
THURSLEY	12.10.65	0.038				0.038	0.0217	0.00
WOODSTOCK	3.3.66	0.056			0.038	0.075	0.0259	0.39
	31.3.66	0.019			0.038	0.067	0.0226	0.68
	28.4.66	0.038			0.038	0.019	0.0208	0.20
	3.10.66	0.056			0.019	0.067	0.0295	0.77
	7.11.66	0.019			0.086	0.058	0.0298	1.08

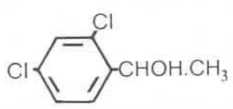
* Significant at 5 % level.
** Significant at 1 % level.

soils and worms. Recoveries of chlorfenvinphos added to untreated samples at the extraction stage averaged 95 % from soils at the 1-5 ppm level and 115 % from worms at the 0.1-0.2 ppm level.

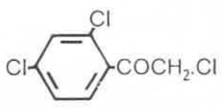
Some extracts were analysed for 2,4-dichloroacetophenone (I) and 1-(2',4'-dichlorophenyl)-ethan-1-ol (II), which are breakdown products of chlorfenvinphos in soil (BEYNON *et al.*, 1967), and also for 2,4-dichlorophenacyl chloride (III), the *in vitro* hydrolysis product of chlorfenvinphos.



(I)



(II)



(III)

TABLE 6

Nematoda

Site	Date of sampling	Animal count as mean log of samples transformed to log (n + 1)					Standard error	Variance ratio (F)
		Untreated control plot	Plots treated as indicated					
			4 lb/ac gran.	4 lb/ac EC	8 lb/ac gran.	8 lb/ac EC		
ALTON	17.5.65	0.067		0.116			0.0806	0.60
	26.8.65	0.075	0.067	0.056			0.0427	0.07
THURSLEY	17.5.65	0.154				0.086	0.0666	0.74
	23.8.65	0.113			0.067	0.132	0.0453	0.56
	12.10.65	0.056				0.019	0.0154	1.00
WOODSTOCK	13.12.65	0.049			0.128	0.105	0.0534	1.00
	27.1.66	0.99			0.038	0.143	0.0357	3.55*
	3.3.66	0.132			0.229	0.202	0.0252	0.78
	31.3.66	0.453			0.386	0.244	0.693	2.03
	28.4.66	0.486			0.302	0.382	0.0737	1.75
	16.6.66	0.105			0.067	0.038	0.0702	1.58
	11.8.66	0.094			0.075	0.015	0.0327	1.89
	3.10.66	0.067			0.019	0.019	0.0288	1.14
	7.11.66	0.038			0.019	0.021	0.0158	0.22

* Significant at 5 % level.

The extracts were analysed for these compounds by gas-liquid chromatography. Full details of the procedure for the analysis for residues of these breakdown products will be published separately.

RESULTS

Tables 2-7 and figures 1-6 summarise the effects of chlorfenvinphos on arthropods, *Annelida* and *Nematoda* as determined from the 2 inch diameter soil cores.

Some groups were represented by too few animals to consider the results from the different sampling dates separately, so those on *Uropodina, Araneida, Poduridae, Chilopoda, Diplopoda, Hemiptera, Lepidoptera* and *Psocoptera* at Woodstock were separately pooled for the treatments and the control (Table 8).

Table 9 gives the wireworms counted by hand-sorting on the two feet square quadrats, and Table 10 counts of the other macro-arthropods in the same samples.

Tables 11 and 12 give results for earthworms from the quadrats and Table 13 the amount of insecticide contained in the earthworms.

Tables 14 and 15 show the results of the experiments with earthworms in boxes, and Table 16 summarises the catches from the pitfall traps.

TABLE 7

Insecta

Site	Date of sampling	Animal count as mean log of samples transformed to log (n + 1): Untreated control plot	Plots treated as indicated: 4 lb/ac gran.	4 lb/ac EC	8 lb/ac gran.	8 lb/ac EC	Standard error	Variance ratio (F)
(1) *Diptera*								
ALTON	17.5.65	0.674		0.394			0.1104	7.73*
	26.8.65	0.425	0.191	0.18			0.0403	6.30**
	13.10.65	0.173		0.184			0.0263	0.01
THURSLEY	17.5.65	0.416				0.135	0.0601	12.69**
	23.8.65	0.199			0.067	0.151	0.0766	2.10
	12.10.65	0.162				0.094	0.0709	1.40
WOODSTOCK	13.12.65	0.33			0.313	0.179	0.067	1.43
	27.1.66	0.45			0.298	0.206	0.0291	2.70
	3.3.66	0.347			0.244	0.327	0.0633	0.64
	31.3.66	0.291			0.288	0.306	0.0769	0.02
	28.4.66	0.235			0.146	0.229	0.0624	0.95
	16.6.66	0.081			0.019	0.067	0.033	0.75
	11.8.66	0.232			0.191	0.141	0.0337	0.81
	3.10.66	0.335			0.454	0.238	0.078	2.55
	7.11.66	0.485			0.523	0.557	0.0696	0.37
(2) *Coleoptera*								
ALTON	17.5.65	0.475		0.310			0.0579	3.38
	26.8.65	0.143	0.108	0.154			0.0593	0.03
	13.10.65	0.154		0.173			0.0536	0.05
THURSLEY	17.5.65	0.39				0.229	0.0512	3.52
	23.8.65	0.108			0.132	0.248	0.0321	1.75
	12.10.65	0.075				0.168	0.053	2.04
WOODSTOCK	13.12.65	0.097			0.086	0.105	0.0237	0.04
	27.1.66	0.099			0.075	0.094	0.0222	0.10
	3.3.66	0.075			0.113	0.056	0.0402	0.57
	31.3.66	0.075			0.056	0.124	0.0447	1.05
	28.4.66	0.105			0.154	0.132	0.0538	0.38
	16.6.66	0.21			0.169	0.162	0.0471	0.59
	11.8.66	0.248			0.24	0.157	0.0438	0.52
	3.10.66	0.289			0.154	0.251	0.05	1.67
	7.11.66	0.313			0.132	0.144	0.0528	4.71*
(3) *Thysanoptera*								
THURSLEY	17.5.65	0.019				0.019	0.0217	0.00
	28.8.65	0.019			0.038	0.019	0.0208	0.25
WOODSTOCK	13.12.65	0.038			0.019	0.019	0.0243	0.25
	11.8.66	0.482			0.229	0.245	0.0383	4.35*
	3.10.66	0.583			0.371	0.208	0.0717	9.68**
	7.11.66	0.569			0.493	0.405	0.0471	1.06
(4) *Protura*								
THURSLEY	23.8.65	0.019			0.03	0.019	0.0211	0.08
WOODSTOCK	13.12.65	0.168			0.067	0.16	0.0714	1.04
	27.1.66	0.094			0.075	0.078	0.0547	0.04
	3.3.66	0.165			0.086	0.17	0.0415	0.50
	31.3.66	0.119			0.119	0.278	0.0584	2.34
	28.4.66	0.124			0.038	0.258	0.0633	5.22*
	16.6.66	0.243			0.067	0.208	0.0582	2.95
	11.8.66	0.165			0.17	0.218	0.0717	0.18
	3.10.66	0.215			0.146	0.265	0.0549	0.73
	7.11.66	0.113			0.21	0.352	0.0531	3.11

* Significant at 5 % level.
** Significant at 1 % level.

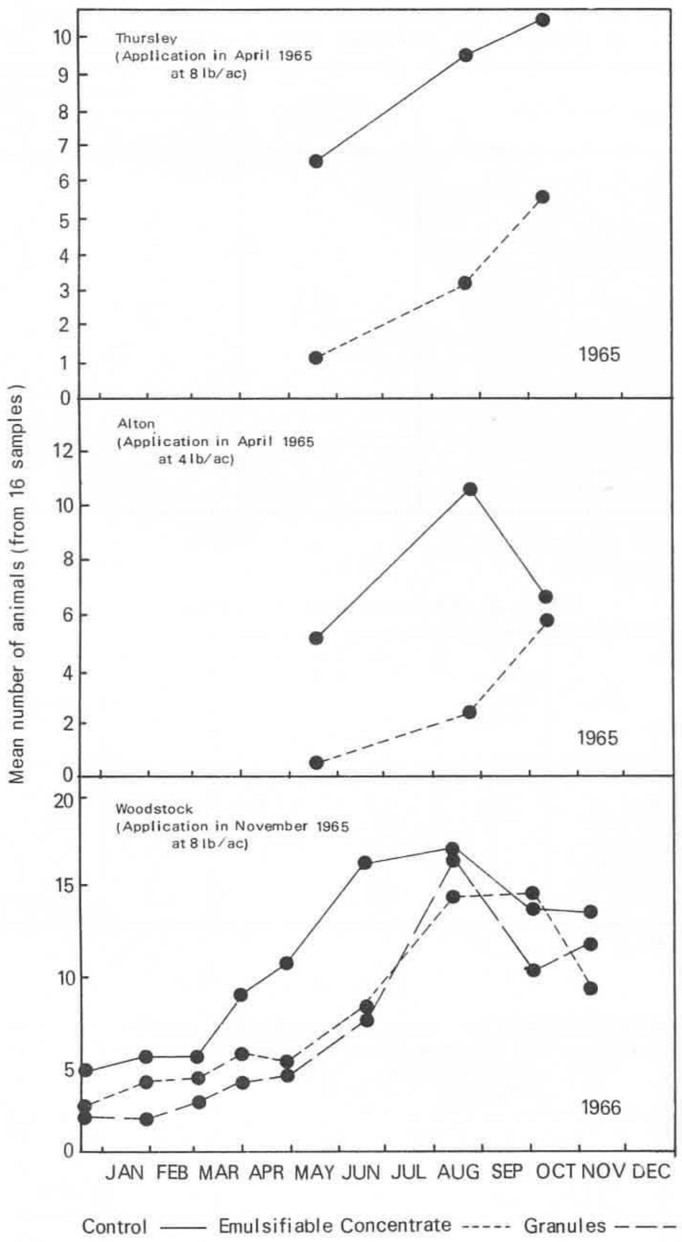


Fig 1 The effect of chlorfenvinphos on soil populations of Mesostigmata

TABLE 8

Numbers of arthropods extracted from Woodstock soil during 12 months after treatment (nine sampling dates)

Animal group	Control (untreated)	Chlorfenvinphos	
		8 lb/ac EC	8 lb/ac gran.
Arachnida			
Uropodina	36	12	4
Araneida	22	12	13
Myriapoda			
Chilopoda	24	18	25
Diplopoda	24	14	19
Collembola			
Poduridae	17	10	10
Insecta			
Hemiptera	58	64	65
Lepidoptera	1	4	3
Psocoptera	9	12	6

TABLE 9

Effect of chlorfenvinphos on wireworms

Treatment	Site	Time after treatment (months)	Mean no. of wireworms per sample		Standard error	Variance ratio (F)	Treated as percentage of control
			Control	Treated			
4 lb/ac EC	Alton	6	4.3	1.8	0.53	11.8**	41.9
4 lb/ac EC	Bethersden	2	6.3	3.1	0.66	5.0**	49.2
8 lb/ac EC	Woodstock	5	3.3	1.1	0.32	10.8**	33.3
8 lb/ac EC	Bethersden	2	6.3	5.1	0.66	5.0**	81.0
8 lb/ac gran.	Woodstock	5	3.3	1.6	0.32	10.8**	48.5
8 lb/ac gran.	Bethersden	2	6.3	3.7	0.66	5.0**	58.7

** Significant at 1 % level.

TABLE 10

Effect of chlorfenvinphos on macro-arthropods five months after treatment

Animal group	Mean number of animals per sample			Standard error	Variance ratio (F)	Treated as percentage of control	
	Control	Treated					
		8 lb/ac gran.	8 lb/ac EC			8 lb/ac gran.	8 lb/ac EC
Diplopoda (millipedes)	0.8	1.1	0.8	1.3	0.4	138	100
Chilopoda (centipedes)	1.9	0.8	0.5	0.5	2.6	42.1	26.3
Coleoptera	3.5	1.8	1.2	0.37	10.5	51.5	34.3
Dipterous larvae	0.6	0.1	0.3	0.2	2.0	16.7	50.0

TABLE 11

Effect of chlorfenvinphos on earthworm populations

Treatment	Site	Time after treatment (months)	Mean no. per sample		Standard error	Variance ratio (F)	Treated as percentage of control
			Control	Treated			
4 lb/ac EC	Alton	6	24.6	13.7	2.7	8.3**	55.7
4 lb/ac EC	Bethersden	2	43.9	36.5	3.1	6.3**	83.1
8 lb/ac EC	Woodstock	5	31.3	23.1	2.6	3.4*	73.8
8 lb/ac EC	Bethersden	2	43.9	28.8	3.1	6.3**	65.6
8 lb/ac gran.	Woodstock	5	31.3	21.8	2.6	3.4*	69.7
8 lb/ac gran.	Bethersden	2	43.9	22.3	3.1	6.3**	50.8

* Significant at 5 % level.
** Significant at 1 % level.

TABLE 12

Effect of chlorfenvinphos on surface and deep populations of earthworms

Treatment	Site	Time after treatment (months)	Surface as percentage of control	Deep as percentage of control
4 lb/ac EC	Alton	6	42.9	66.5
4 lb/ac EC	Bethersden	2	90.8	68.3
8 lb/ac EC	Woodstock	5	67.6	79.2
8 lb/ac EC	Bethersden	2	55.9	84.4
8 lb/ac gran.	Woodstock	5	58.4	79.7
8 lb/ac gran.	Bethersden	2	52.7	46.7

Finally, Tables 17-20 show the amount of chlorfenvinphos remaining in the soil. In these tables, the results are not corrected for the percentage recovery of the insecticide but are corrected for the blank values obtained with untreated controls.

TABLE 13

Analysis of earthworms for chlorfenvinphos residues (field experiments)

Treatment	Site	Time after treatment (months)	Pesticide residues (ppm)	
			Earthworms from treated soil	Earthworms from untreated soil
4 lb/ac EC	Thursley	6	0.02	0.01
8 lb/ac EC	Thursley	6	0.02	0.01
4 lb/ac EC	Alton	6	0.02	0.01

TABLE 14

The analysis of soils and earthworms for chlorfenvinphos residues (experiments in boxes)

Soil type	Time interval between application and sampling (weeks)	Chlorfenvinphos residues in dry soil** (ppm)	Chlorfenvinphos residues in earthworms (ppm)	Earthworms recovered alive	
				Untreated soil	treated soil
Peat	0	12	—	—	—
	4	10	—	13	13
	10	6.7	< 0.02	14	14
	21	6.3	< 0.02*	7	14
Sandy loam ..	0	6.7	—	—	—
	4	1.4	< 0.02	13	14
	10	0.55	< 0.02	14	13
	21	0.22	< 0.02	11	11
Medium loam.	0	7.6	—	—	—
	4	3.0	< 0.02	13	12
	10	1.1	< 0.02	10	13
	21	0.39	< 0.02	14	13

* Chlorfenvinphos residues were just detectable in this case: in all other earthworms chlorfenvinphos could not be detected.

** Moisture contents (% dry weight of soil).

Peat	105-130
Sandy loam	12-19
Medium loam	26-34

TABLE 15

The analysis of soils and earthworms for residues of chlorfenvinphos metabolites (experiments in boxes)

Soil type	Time interval between application and sampling (weeks)	2,4-Dichloroacetophenone residues (ppm) *	
		Dry soil	Earthworms
Peat	0	NA	NA
	4	NA	< 0.02
	10	0.04	< 0.02
	21	0.04	< 0.02
Sandy loam	0	NA	NA
	4	NA	< 0.02
	10	0.07	< 0.02
	21	0.05	< 0.02
Medium loam	0	NA	NA
	4	NA	< 0.02
	10	0.13	< 0.02
	21	0.04	< 0.02

* Blank values with untreated control soils : Peat 0.01, Sandy loam 0.01, Medium loam 0.01, Earthworms 0.02. NA Not analysed.

2,4-Dichlorophenacyl chloride and 1-(2',4'-dichlorophenyl) ethan-1-ol could not be detected in the earthworms and soils that were analysed (the limit of detectability here was near 0.05 ppm).

TABLE 16

Pitfall trap catches

Treatment	Number of animals trapped from each animal group												
	Coleoptera							Diptera	Hymenoptera			Diplopoda	Araneida
	Elateridae	Halticidae	Carabidae	Staphylinidae	Rhyncophora	Various	Total		Ants	Others	Total		
Control	3	6	12	62	—	3	86	15	27	2	29	3	14
4 lb/ac EC	6	10	8	58	2	4	88	13	10	11	21	—	9
8 lb/ac gran.	17	22	17	35	—	2	93	12	12	2	14	1	15
8 lb/ac EC	5	7	6	20	—	1	39	9	9	3	12	—	13

TABLE 17

The analysis of soils for residues of chlorfenvinphos

Site of trial: Thursley, Surrey

Application	Dosage level (ai)	Time interval between application and sampling (weeks)	Chlorfenvinphos residues in dry soil (ppm)	
			Results	Mean
Chlorfenvinphos Soil application of 50 % EC incorporated.	8 lb/ac	11	1.9 1.8	1.9
		19	2.3 2.3	2.3
Untreated controls	—	11	0.14 0.34	0.24
		19	0.11 0.11	0.11

Plot size: 10 ft × 20 ft.
Replication: 4 times.
Date of application: 13th April, 1965.
Soil type: sandy loam.
Prevailing weather: wet and sunny (showery rain).
Mean recovery of chlorfenvinphos untreated soil was 96 % at 1 ppm level.

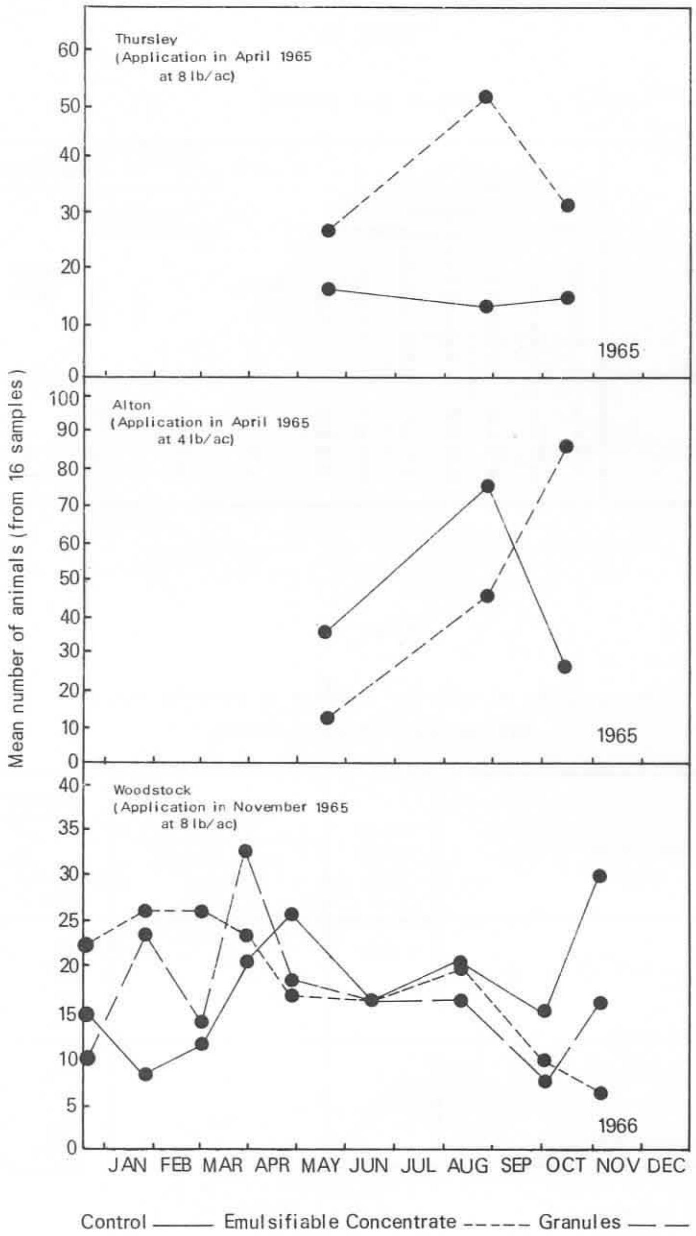


Fig 2 The effect of chlorfenvinphos on soil populations of Oribatidae

TABLE 18

The analysis of soils for residues of chlorfenvinphos

Site of trial: Alton, Hampshire

Application	Dosage level (ai)	Time interval between application and sampling (weeks)	Chlorfenvinphos residues in dry soil (ppm)	
			Results	Mean
Chlorfenvinphos .... Soil application of 50 % EC incorporated.	4 lb/ac	11	2.1 2.2	2.2
		19	2.0 1.4	1.7
Chlorfenvinphos .... Soil application of 10 % gran incorporated.	4 lb/ac	19	3.6 4.0	3.8
Untreated controls	—	11	0.06 0.05	0.06
		19	< 0.02 < 0.02	< 0.02

Plot size: 10 ft × 20 ft.
Replication: 4 times.
Date of application: 13th April, 1965.
Soil type: loam overlying clay.
Prevailing weather: showery soon after application.
Mean recovery of chlorfenvinphos from untreated soil was 90 % at 1 ppm level.

DISCUSSION

The effects of chlorfenvinphos on the population of soil invertebrates were complex, but consistent at the different experimental sites. Possibly the effects may be similar for most agricultural soils.

Families of *Collembola* did not all react similarly to the insecticide. Numbers of *Isotomidae* were diminished in all the experiments, but recovered after six to seven months (fig. 4); the decrease was greatest at Woodstock where there were most *Isotomidae* before the insecticide was applied. Effects on *Onychiuridae* were less definite (fig. 3). At Alton, numbers were greatly diminished at first but after about four months they exceeded those in untreated soil. At Thursley, numbers diminished slightly to begin with but after five months there were more in the treated plots than in the untreated plots; at Woodstock, numbers in treated plots never exceeded those in untreated soil.

At all sites, there were more *Entomobryidae* in the treated plots than in the untreated plots even at six months after application (fig. 5). Such increases have also been reported by SHEALS (1956) and EDWARDS (*et al.* 1960

and 1965) in soils treated with other insecticides. The increase in numbers in response to DDT in soil was attributed to the death of the parasitic mites that are among the chief predators of *Collembola*. This may have happened in the present experiments, as numbers of predatory mites were much diminished in all of them (fig. 1).

TABLE 19

The analysis of soils for residues of chlorfenvinphos

Site of trial: Woodstock, Kent

Application	Dosage level (ai)	Time interval between application and sampling (weeks)	Chlorfenvinphos residues in dry soil (ppm)	
			Results	Mean
Chlorfenvinphos .... Soil application of 50 % EC incorporated.	8 lb/ac	4	3.4 3.1	3.3
		11	1.9 2.2	2.1
		15	0.66 —	0.66
		23	0.42 0.41	0.42
		30	0.14 0.13	0.14
		38	< 0.01 < 0.01	< 0.01
Chlorfenvinphos .... Soil application of 10 % gran incorporated	8 lb/ac	4	7.9 6.5	7.2
		11	4.7 4.7	4.7
		15	2.5 3.2	2.9
		23	1.5 1.6	1.6
		30	0.13 0.14	0.14
		38	0.01 0.01	0.01
Untreated control	—	4	0.06 0.07	0.07
		11	0.09 0.10	0.10
		15	0.01 < 0.01	0.01
		23	0.01 < 0.01	0.01
		30	< 0.01 < 0.01	< 0.01
		38	< 0.01 < 0.01	< 0.01

Plot size: 12 ft × 12 ft.
Replication: 4 times.
Date of application: 15th November, 1965.
Soil type: clay loam.
Mean recovery of chlorfenvinphos from untreated soil was 90 % at 1 ppm level.

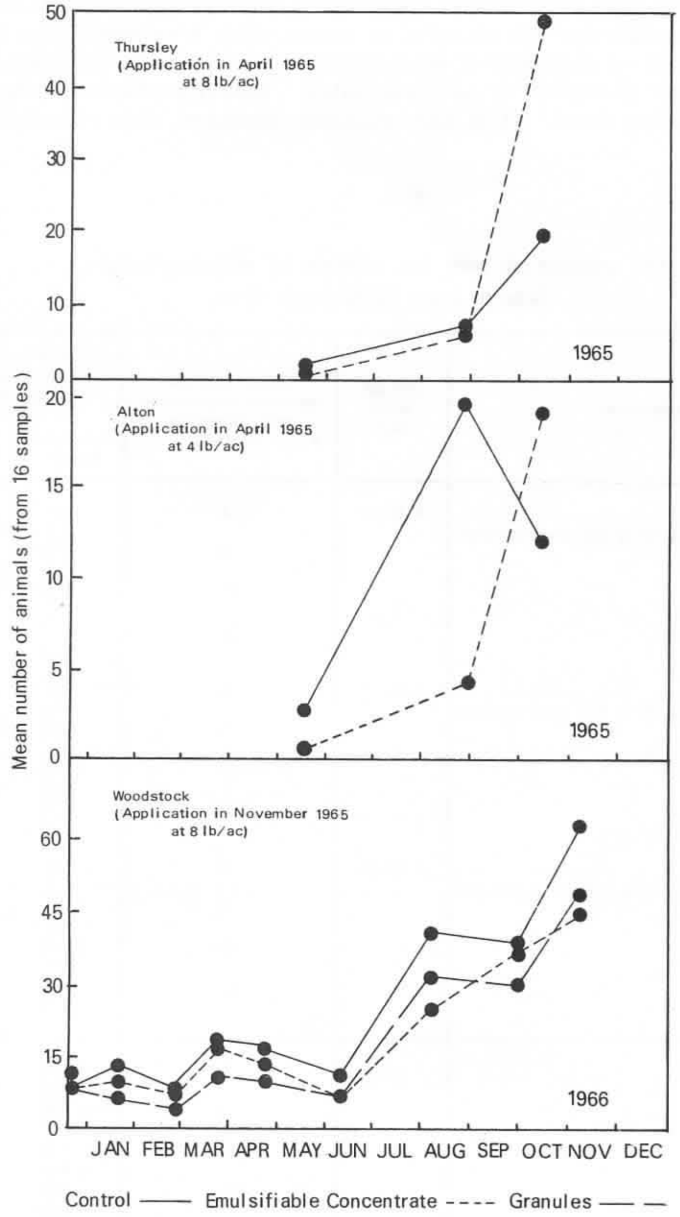


Fig 3 The effect of chlorfenvinphos on soil populations of Onychiuridae

Numbers of *Collembola* increase after soil has been treated with other insecticides (SHEALS 1956, EDWARDS 1960 *et al.* and 1965), but in our experiments there was also a consistent increase in numbers of oribatid mites that was still noticeable for six months or more after treatment (fig. 2). This may be explained by numbers of *Mesostigmata* being greatly lessened, because they are possibly predators of oribatid mites. This hypothesis can be verified only by laboratory tests. It is also possible, however, that chlorfenvinphos,

TABLE 20

The analysis of soils for residues of chlorfenvinphos

Site of trial: Bethersden, Kent

Application	Dosage level (ai)	Time interval between application and sampling (weeks)	Chlorfenvinphos residues in dry soil (ppm)	
			Results	Mean
Chlorfenvinphos .... Soil application of 50 % EC incorporated.	4 lb/ac	4	1.1 1.5	1.3
		9	0.29 0.31	0.30
		15	< 0.01 < 0.01	< 0.01
		19	< 0.01 < 0.01	< 0.01
Chlorfenvinphos .... Soil application of 50 % EC incorporated.	8 lb/ac	4	4.4 3.8	4.0
		9	0.82 0.87	0.85
		15	0.01 0.01	0.01
		19	0.01 0.01	0.01
Chlorfenvinphos .... Soil application of 10 % gran incorporated.	8 lb/ac	4	2.6 2.3	2.5
		9	1.3 1.4	1.4
		15	0.01 0.01	0.01
		19	< 0.01 < 0.01	< 0.01
Untreated control	—	4	< 0.01 < 0.01	< 0.01
		9	< 0.01 < 0.01	< 0.01
		15	< 0.01 < 0.01	< 0.01
		19	< 0.01 0.02	0.02

Plot size: 12 ft × 12 ft.
Replication: 4 times.
Date of application: 17th March, 1966.
Soil type: clay with loam.
Mean recovery of chlorfenvinphos from untreated soil was 95 % at 1 ppm level.

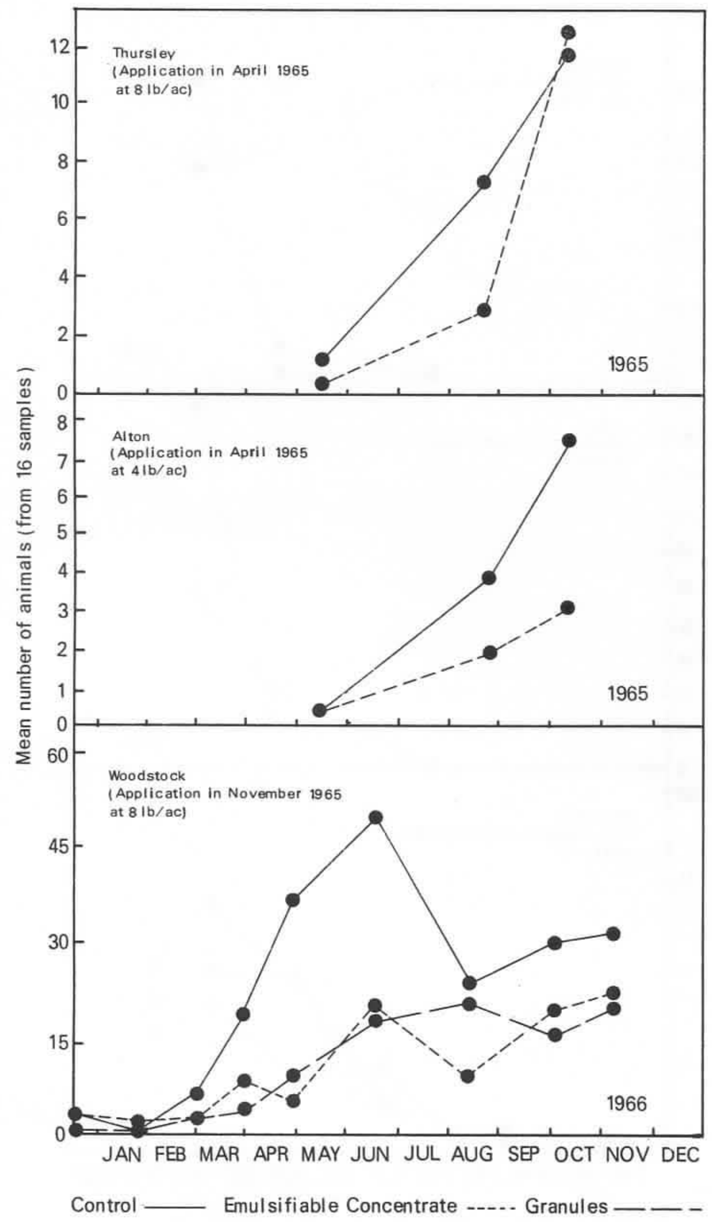


Fig 4 The effect of chlorfenvinphos on soil populations of Isotomidae

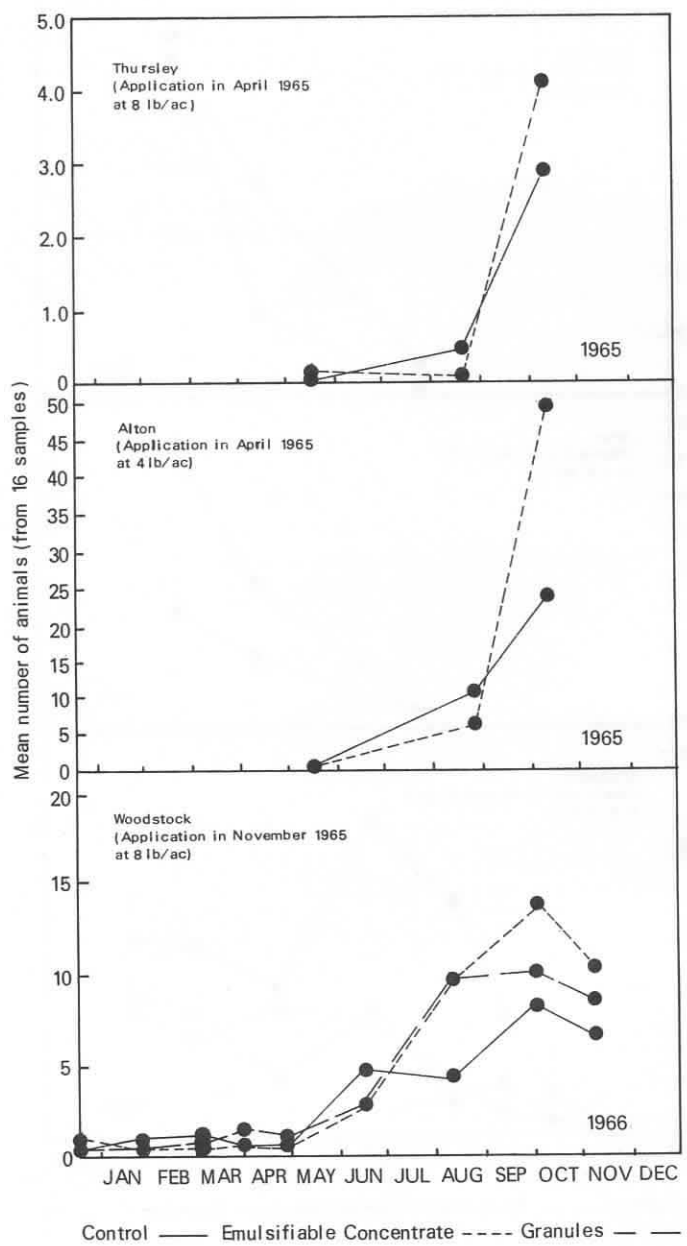


Fig 5 The effect of chlorfenvinphos on soil populations of Entomobryidae

at the same application rates, stimulates reproduction of *Collembola* or *Oribatidae*.

Chlorfenvinphos seemed not to be very lethal to *Coleoptera* (mostly weevils, chafers and wireworms) (Table 7). Populations of wireworms were never decreased by more than 70 % (Table 9). Other *Coleoptera* in the two inch diameter cores were too few to assess any significant decrease in numbers after the treatments. Results for *Coleoptera* from the pitfall traps were inconclusive, probably because the beetles moved from plot to plot. The quadrat sampling at Woodstock showed that numbers of *Coleoptera* diminished more than 50 %, but this was in response to doses of 8 1b/ac, a larger dose than would normally be used in commercial agricultural practice.

Counts of the dipterous larvae extracted from the two inch diameter cores showed that chlorfenvinphos consistently lessened their populations to a half or fifth during the first few months after treatment (fig. 6); the quadrat sampling at Woodstock showed a similar decrease (Table 10). The drastic effects of chlorfenvinphos on dipterous larvae were not unexpected as previous field evaluations showed it to be more active against dipterous than against other arthropod pests. As the percentage of insecticide remaining in the soil diminished, so the control of dipterous larvae also diminished. This differed from the effects on micro-arthropods, with which the effects on population were apparent long after the residues had almost disappeared.

Chlorfenvinphos had little effect on the other arthropods (Table 8), and the only group it appreciably affected was the *Pauropoda* (Table 4). These tiny animals are usually sensitive to insecticides (EDWARDS 1965) but their food and ecological importance are unknown.

The quadrat sampling allowed the effect of chlorfenvinphos on earthworms dwelling in the top 3-4 inches to be distinguished from that on the deeper-dwelling worms (Table 12). Although there were exceptions, the surface dwelling forms were usually affected more than the deeper forms. It is unlikely that the initial rotovation of the soil after treatment buried much insecticide deeper than four inches (EDWARDS, 1966) and whilst no information is available at present concerning the leaching of chlorfenvinphos, it is considered unlikely that it penetrates much below the depth of cultivation. Thus deeper dwelling forms that were killed may have picked up insecticide when burrowing to the surface. The earthworm population was at most halved, and the effects were not drastic because cultivations often kill many more of the surface-dwelling worms. Most soils that are regularly cultivated contain fewer worms than pasture or undisturbed soils.

The results with earthworms in boxes differed from those in the field. In the boxes, chlorfenvinphos at 6 ppm produced no detectable mortality of the worms within 21 weeks of treatment (Table 15). Possibly the worms are more active in the field than in boxes. It is also possible that the pesticide was distributed less uniformly in the field than in the boxes so that the worms in the field could have been exposed to local concentrations of chlorfenvinphos very much in excess of 6 ppm. Tissues of earthworms from the boxes and field were analysed for chlorfenvinphos and its metabolites and not more than 0.02 ppm was found in any specimen. These analyses were done because other insecticides, particularly aldrin and drieldrin, have been reported to occur in worm tissues, in appreciable amounts, and it has been

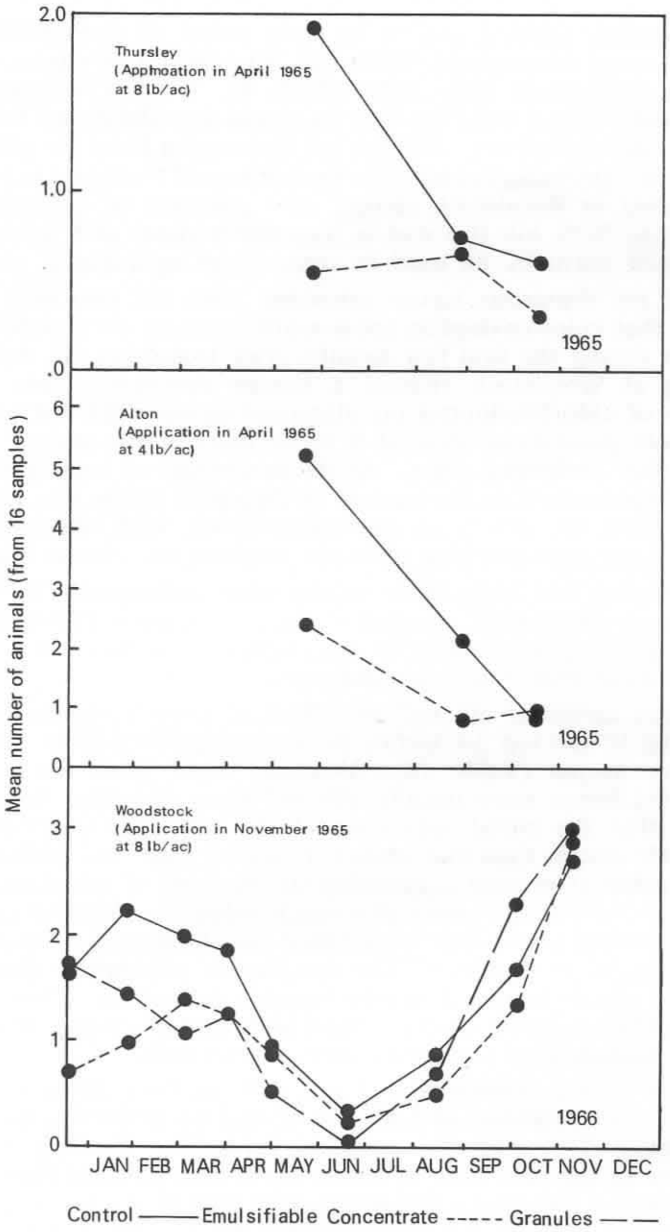


Fig 6 The effect of chlorfenvinphos on soil populations of Diptera

claimed that this poisons birds. The absence of appreciable quantities (< 0.02 ppm) of chlorfenvinphos in the worms makes such a hazard unlikely with this compound.

The effects of chlorfenvinphos are so varied that it is necessary to summarise the beneficial and deleterious aspects. The insecticide controls *Diptera* well, but *Coleoptera* less so, and it has little effect on *Symphyla*. Predatory centipedes and parasitic mites are affected but they are less important than predatory *Coleoptera*, which are not greatly affected.

Soil invertebrates, especially *Collembola*, *Acarina*, *Diplopoda*, dipterous larvae and earthworms, help to break down plant material and organic matter in the soil. The numbers of *Collembola* increase after treatment with chlorfenvinphos, there is little effect on *Diplopoda*, and dipterous larvae and earthworms are depleted. EDWARDS and HEATH (1963) showed that in arable soils earthworms probably account for about 60 % of the initial breakdown of plant material and other invertebrates about 40 %, so the increased number of *Collembola* may complement the decreased numbers of earthworms.

After nine months, some groups of soil invertebrates were still affected by the insecticide, but after ten months, almost all the chlorfenvinphos applied at Woodstock had disappeared, and from the results it seemed unlikely that effects on soil fauna would persist for longer than one year.

SUMMARY

The effects on soil invertebrates were studied of the organo-phosphorus insecticide chlorfenvinphos [Birlane, diethyl 1-(2',4'-dichlorophenyl)-2-chlorovinyl phosphate, previously known as compound SD 7859 and GC 4072]. It increased the numbers of *Collembola* and sometimes *Oribatidae* and decreased the numbers of *Mesostigmata*. The insecticide controls *Diptera* well but numbers of *Coleoptera* (mostly wireworms, chafers and weevils) were never decreased by more than 70 %. As the amount of the insecticide remaining in the soil diminished, so the effect on dipterous larvae diminished. Chlorfenvinphos has little effect on *Symphyla* and *Diplopoda*.

From a fifth to a half of the earthworms in the field were killed and more surface-dwelling forms were killed than deeper-dwellers. Even after 21 weeks in soil treated with 6 ppm of chlorfenvinphos, earthworms had no more than 0.02 ppm of insecticide and its metabolites in their tissues.

Other groups of invertebrates were not drastically affected.

Effects of chlorfenvinphos on soil fauna seem unlikely to persist for more than one year.

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